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Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes

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Abstract

A field study was conducted on three Swiss farms to investigate the efficacy of *Duddingtonia flagrans* against naturally acquired infections of gastrointestinal nematodes in adult dairy sheep. On each farm the ewes were divided into two equal groups. One group received *Duddingtonia* during a period of 4 months at a daily dose rate of 10^6 chlamydospores per kilogram body weight, the second group acted as controls. At an overall moderate infection level in all farms *D. flagrans* did not have a significant effect on the observed parasitological parameters with the exception of a significantly reduced herbage infectivity in one farm. In contrast, the results from faecal cultures indicated a mean suppression of larval development during the fungus-feeding period between 82, 89 and 93% on the three farms, respectively. The discrepancy observed between the fungus efficacy in coprocultures and on pasture, which was also observed in several other studies deserves further research.

Keywords: Dairy sheep; Trichostrongyles; Biological control; *Duddingtonia flagrans*

1. Introduction

Infection with gastrointestinal nematodes causes economic losses to the sheep industry. For more than 50 years, control of gastrointestinal nematodes in sheep has relied almost entirely on the use of anthelmintics. As a result the prevalence of parasite strains resistant to anthelmintics, particularly benzimidazoles, is increasing (Hertzberg and Bauer, 2000; Jackson and Coop, 2000). Avermectins and imidazothiazoles are not registered for lactating sheep, therefore no effective nematocidal compounds are currently available for use in dairy sheep. Anthelmintic resistance and the desire to produce agricultural commodities free from chemical residues have resulted in the development of innovative parasite control strategies. Recent and ongoing studies are investigating the development of nematode-resistant breeds (Woolaston and Baker, 1996), the use of pasture management systems (Barger, 1999), vaccination (Newton and Meeusen, 2003), plants containing condensed tannins (Min and Hart, 2003) and the potential of nematophagous fungi as biological control agents. Research on nematophagous fungi with potential as biological control candidates have focused on the nematophagous microfungus *Duddingtonia flagrans* (reviewed by Waller and Faedo, 1996; Larsen, 2000). Studies by Larsen et al. (1991) demonstrated that *D. flagrans* is well suited for gut passage because it produces thick-walled chlamydospores. These spores germinate in freshly discharged dung, spread rapidly and capture infective larvae of parasites before they

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migrate onto herbage. The high potential of *D. flagrans* as a biological agent against free-living stages of bovine trichostrongylid nematodes has been documented in several field studies (Grønvold et al., 1993; Larsen et al., 1995; Nansen et al., 1995). Among the various studies with small ruminants some are reporting on the effect of fungal treatment in adult sheep and goats grazing on naturally infested pastures (Fontenot et al., 2003; Wright et al., 2003; Waller et al., 2004, 2006; Eysker et al., 2006; Gomez-Rincon et al., 2006; Maingi et al., 2006). Although the conclusions from most of these studies were encouraging, it seems that treatments similar to those that proved successful in cattle did not consistently exhibit the same beneficial effects in small ruminants. Little information is available on the effect of treatment of adult dairy sheep with *D. flagrans*-spores on naturally infested pasture. Therefore, the aim of the present study was to examine the potential of *D. flagrans* as biological control agent against GIN in adult dairy sheep kept under field conditions in the Swiss midland and pre-alpine region.

2. Materials and methods

2.1. Experimental design

Three dairy sheep farms located in north-east Switzerland with different levels of gastrointestinal nematode infections were selected for the study. A summary of the trial periods, period of fungus-feeding, number and breeds of sheep, stocking rate and number of paddocks used in each farm is given in Table 1. On each farm adult (2–6 years old) dairy sheep were randomly assigned to two groups (A and B). Prior to turnout the pasture was divided by electrical fencing into equally sized paddocks of similar vegetation type. The two groups grazed separately and were rotated between their paddocks synchronously. The areas which were left ungrazed early in the season were cut for hay, and were incorporated stepwise into the grazing cycle. From turnout for the following 4 months, millet grain to which *D. flagrans* had been cultured (Troll A isolate) was given once a day to ewes of group A at a dose of 10^6 chlamydospores of *D. flagrans* per kilogram of body weight. The fungal-millet material was mixed into the concentrate and offered in a feeding trough during milking. The control group B received an equal amount of concentrate without the addition of the fungal-millet material. Ewes were observed to ensure that all supplement was consumed. Herbage, individual faecal and blood samples were collected at 4-weekly intervals. Animals were inspected daily and detailed examinations of each animal were made on the sampling dates.

2.2. Parasitological analyses

Faecal samples were collected from the rectum of the animals. To determine parasite egg counts modified McMaster method (Schmidt, 1971) was used. Eggs other than strongyles eggs were categorized separately. Cultivation of infective larvae was performed according to Henriksen and Korsholm (1983). Every two months the faecal samples were examined for *Fasciola hepatica* eggs using a modified sedimentation method (Boray and Pearson, 1960) and for larvae of large and small lungworms using the Baermann method (Rommel et al., 2000). In order to determine the number of parasitic nematode larvae per kilogram of grass, samples were collected at 4-weekly intervals from all the paddocks grazed by the fungi-fed and control ewes, respectively. The L3 were processed and counted according to the method of Sievers Prekehr (1973) and modified by Hertzberg et al. (1992). Mean pasture contamination with L3 was calculated considering the size of each paddock for groups A and B.

Blood samples from jugular veins were taken for serum pepsinogen and haematocrit determination. The serum pepsinogen concentration was estimated using the method described by Berghen et al. (1987) and the haematocrits were determined using the microhaematocrit method. At every sampling date each animal was

Table 1
Summary of farms and study design

Farm	Height above Sea level (m)	Sheep breed	Trial period	Period of feeding fungus	Number of sheep	Sheep per hectare	Number of paddocks per group
I	1050	East Friesian	30 April–22 October	30 April–20 August	36 (18[a]; 18[b])	5.1[a]; 5.1[b]	7
II	450	East Friesian	22 April–04 November	22 April–12 August	74 (36[a]; 38[b])	16.4[a]; 17.3[b]	2
III	900	Lacaune	29 April–29 October	29 April–20 August	51 (27[a]; 24[b])	19.3[a]; 17.1[b]	2

[a] Duddingtonia group.
[b] Control group.

tested for anaemia using the FAMACHA© chart (van Wyk and Bath, 2002). The color of the ocular mucous membranes of each animal was examined and classified into one of five categories according to the FAMACHA© chart: 1 = red, non-anemic; 2 = red-pink, non anemic; 3 = pink, mildly-anemic; 4 = pink-white, anemic; 5 = white, severly anemic.

2.3. Meteorological data

For the whole trial period daily temperature and rainfall data were obtained from the national weather service. The closest measuring points were chosen for each farm ranging between 10 and 25 km distance to the experimental pastures.

2.4. Statistical analyses

The differences between farms and groups were determined by maximum likelihood techniques assuming negative binomial distributions. The maximum likelihood value of a model with separate means for treated and control groups was compared to the likelihood of a model with a single means with control and treated animals in one group and significant differences were based on the likelihood ratio test (Torgerson et al., 2003). The differences for larval herbage counts between the fungus-treated and the control group were tested using the Wilcoxon signed rank test. Serum pepsinogen levels were correlated with partial e.p.g's for the various strongyle worm genera. The sums of partial e.p.g. for different combinations of genera were also correlated with the serum pepsinogen levels. The association between the partial e.p.g. (only the part concerning *H. contortus* was considered), haematocrit and FAMACHA© scores were analysed using the Spearman rho-test.

3. Results and discussion

Temperatures for the trial period were warmer than the 30-year average (1961–1990), resulting in higher mean values of approximately 3 °C. Total precipitation was slightly lower on all farms compared with the 30-year average (farm I: 833 versus 961 mm; farm II: 588 versus 665 mm; farm III: 883 versus 640 mm). In all farms a maximum of 3 weeks without any rain was recorded (Table 2). In combination with regular generation of dew during nights, conditions were regarded as sufficient for larval migration onto pasture.

At an overall moderate infection level the data indicate that the treatment of dairy ewes with *D.*

Table 2
Total precipitation (in mm) per week for farms I–III

	April			May			June			July			August			September			October		
Farm I	32	10	17	13	45	71	44	34	22	57	12	0	65	32	0	44	30	16	0	30	18	62	18	22	23	15	99	18	18	25
Farm II	19	10	4	10	26	18	26	25	3	23	6	2	15	39	0	35	39	12	0	40	0	50	7	13	5	22	81	45	10	14
Farm III	30	20	17	12	33	34	47	49	18	69	83	20	14	41	0	67	67	4	0	13	0	67	19	15	3	36	88	63	9	18

Table 3
Nematode faecal egg counts (eggs per gram) of ewes in control and fungus treatment groups on three commercial farms (farm I–III)

	Farm I	
	Control (e.p.g.)	Fungus (e.p.g.)
30 April	242	72
27 May	50	81
24 June	116	33
22 July	165	233
19 August	200	525
16 September	130	241
22 October	231	103
	Farm II	
	Control (e.p.g.)	Fungus (e.p.g.)
23 April	42	62
20 May	74	36
17 June	78	25
15 July	91	119
12 August	211	165
09 September	410	311
07 October	643	384
04 November	426	250
	Farm III	
	Control (e.p.g.)	Fungus (e.p.g.)
07 May	776	415
10 June	200	713
08 July	930	121
05 August	1426	171
02 September	558	352
30 September	292	163
29 October	276	244

No significant differences ($p > 0.05$) were detected between the faecal egg counts of the control and treatment group on any date.

Table 4
Total number of infective third-stage trichostrongyle larvae (L3) per kg dried herbage on three commercial farms

	Farm I	
	Control (L3/kg)	Fungus (L3/kg)
30 April	250	250
27 May	139	119
24 June	119	194
22 July	158	66
19 August	197	437
16 September	657	428
22 October	65	161
	Farm II	
	Control (L3/kg)	Fungus (L3/kg)
23 April	0	0
20 May	149	75
17 June	64	14
15 July	40	7
12 August	39	39
09 September	65	39
07 October	300	206
04 November	166	123
	Farm III	
	Control (L3/kg)	Fungus (L3/kg)
07 May	0	0
10 June	111	207
08 July	53	31
05 August	1184	377
02 September	20	27
30 September	82	29
29 October	153	350

Differences were significant only for farm II ($p < 0.05$). Only on farm II was there evidence of a significant reduction in pasture infectivity resulting from the fungus-treatment ($p < 0.05$).

flagrans spores did not result in a significant reduction of the trichostrongyle egg counts in faeces when compared with the controls in each of the three farms (Table 3). Only on farm II was there evidence of a significant reduction in pasture infectivity resulting from the fungus-treatment ($p < 0.05$) (Table 4). Based on faecal cultures *Teladorsagia circumcincta* and *H. contortus* were the dominating species in all 3 farms. Eggs of *Trichuris ovis*, *Moniezia* sp. and *Dicrocoelium dendriticum* were detected in very small numbers and *Fasciola hepatica* eggs were absent in the faeces during the entire observation period. The moderate infection level with trichostrongyles was also reflected by the haematocrit values, FAMACHA© scores and pepsinogen levels, with the latter never exceeding 1000 mU as the group mean. Eighty percent of the haematocrit values ranged between 28 and 34%. The overall frequency of all measured FAMACHA© scores 1–4 was 31.2, 56.4, 12.2 and 0.1%, respectively. The correlation between *H. contortus* e.p.g. and haematocrit was negative ($r = -0.17$) and significant ($p < 0.01$). FAMACHA© score values correlated negatively with the haematocrit ($r = -0.16$; $p = 0.01$) confirming this technique as a reliable tool for estimating the extent of *Haemonchus*-induced pathogenicity.

None of the farms had a history of significant problems with GIN infections and the overall infection levels were considered to be within the normal range of these farms. Nevertheless, the above average temperatures and below average humidity conditions will have contributed to a moderate development of pasture infectivity.

Previous field studies using *Duddingtonia* in sheep have yielded conflicting results. In the first published study with sheep Githigia et al. (1997), using tracer lambs, concluded that *D. flagrans* may limit the build up

of pasture contamination in the late grazing season. However, an early season outbreak of nematodirosis occurred in both fungus-treated and untreated lambs, indicating that *Nematodirus*-species are likely to be refractory to the *Duddingtonia*-strategy. Knox and Faedo (2001) demonstrated reduced faecal egg counts in *Duddingtonia*-treated lambs, but observed substantial differences between identically treated groups. In a Swedish transhumance grazing system Waller et al. (2004) described a positive effect of the *Duddingtonia*-application to ewes on lamb marketing at the end of the season. These benefits were associated with significantly lower worm burdens in some groups of experimental and tracer lambs in the *Duddingtonia*-system, but not with lower faecal egg counts. Similar results have been reported from an experiment where ewes were fed spores over the entire grazing period (Fontenot et al., 2003). Although faecal egg output differed not significantly between animals of both groups, worm burdens of tracers grazing on *Duddingtonia*-treated pastures were reduced. Larsen et al. (2005) could not observe a significant parasitological benefit in fungus-treated lambs, although there was evidence for a better productivity in these animals. In contrast Eysker et al. (2006) were not able to find any significant benefit in ewes or lambs after ewes had been fungus-treated over a period of 9 weeks. Similar to the present study faecal egg counts and pasture larval counts did not differ between groups, although the trapping activity of *D. flagrans* was clearly visible in the faecal cultures. Development of haemonchosis in all groups of lambs indicated that sufficient amounts of infective larvae had been able to bypass the trapping structures.

The majority of the experiments discussed above were similar with respect to the underlying spore dose and the varying spectrum of parameters positively influenced by the fungus-treatment clearly deserves further attention. In total, the results of experiments performed with sheep are clearly less convincing when compared with a range of field studies with first season grazing cattle (Nansen et al., 1995; Fernandez et al., 1999; Larsen, 1999) in which treated animals constantly exhibited lower trichostrongyle egg excretion, associated with improved liveweight performances in all studies.

In contrast to the situation on the experimental pastures the nematode-destroying effect of *D. flagrans* in the present study was clearly apparent in the larval cultures performed during the 4 months fungal dosing period. Larval counts from faecal cultures recorded from the fungi-fed groups were reduced between 77 and 100% (mean 93%) for farm I, between 65 and 93% (mean 82%) for farm II and between 80 and 95% (mean 89%) for farm III when compared with those of the control groups. There was no indication for a selective reduction of single genera. After fungus-feeding was terminated similar recovery rates were seen in the fungus and the control groups. The substantial trapping efficacy documented by the larval cultures confirmed the successful intake of the spore material during the milking period as reported by the farmers. In a study with calves, performed at the same time as the present experiment, the nematode destroying effect of *D. flagrans* was not limited to faecal cultures but was also reflected in the pasture larval counts (data not shown) indicating an accelerated fungal trapping activity in the cattle faeces. A possible explanation for this observation could be differences in the structure of sheep and cattle faeces which may have important impacts on fungal development and/or activity. Humid conditions in cattle faeces are likely to favour fungal growth more than conditions in the pelleted faeces of sheep and goats. Especially during the summer sheep faeces may dry out more quickly and mycelial growth may be retarded (Grønvold et al., 1999). This view is supported by our observation that *D. flagrans* showed a better trapping capacity in sheep faeces which had been formed into little pads of 30–120 g when compared with the same amounts of regular pelleted faeces (Hertzberg, unpublished data). This effect is likely to be even more important for faeces from small lambs compared with ewes.

Many dairy sheep are kept in organically managed farms and are therefore an important target of alternative control measures directed against GIN. Options for anthelmintic treatments are limited as there are few products licensed for use in such animals and all of them are restricted to the dry period. The daily spore application was well accepted by the farmers in the present study, but this approach is yet not regarded as practical for meat farmers, who do not handle their animals on a daily basis. In order to increase the efficacy of *D. flagrans* in sheep future studies should also focus on improved strategies for spore application. Beside the development of long acting spore release devices (Waller et al., 2001a), incorporation of spores into urea/molasses blocks offer potential deployment options for *D. flagrans* especially in the developing world (Waller et al., 2001b).

Today, the successful establishment of the *Duddingtonia*-strategy in sheep still seems to be a substantial higher challenge compared with cattle and currently their efficacy cannot be regarded as sufficient on its

own. Therefore, the potential of this approach to control GIN infections in small ruminants under different grazing conditions needs to be further evaluated considering all factors that could improve this strategy generating a component to be incorporated in an integrated control approach.

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